REMARKS

Claims 1, 2 and 4-41 are pending in the application. Claims 23-41 are withdrawn from consideration as being drawn to a non-elected invention. Claims 5-14 and 20 are withdrawn from consideration as being drawn to a non-elected species. Claims 1 and 4 have been amended to better clarify what Applicants believe to be the invention. Support for the amendments to claim 1 can be found throughout the specification, but particularly on page 18, lines 6-11 and in original claim 4. Support for the amendment to claim 4 can be found throughout the specification but particularly on page 45, lines 21-23, continuing on to page 46, lines 1-4. Furthermore, portions of the language from claim 4 have been incorporated into claim 1 to better clarify the invention. Accordingly, claims 1, 2, 4, 15-19, 21 and 22 remain under consideration.

Applicants' representatives would like to express their sincere appreciation for the courteous and constructive telephonic conference held with Examiner Canella on November 8, 2005 as related to the claims under consideration. As noted in that conversation, Applicants' representatives presented to Examiner Canella proposed claim amendments and discussed the differences between the claimed invention and the cited references. Examiner Canella agreed that insertion of these particular recitations into the claims should be sufficient to differentiate the present invention from the cited art. Accordingly, these claim amendments have been made and are included in the instant response.

Rejection under 35 U.S.C. §112, first paragraph

The Examiner has rejected claims 4 and 14-18 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The Examiner notes that claim 4 has been amended to incorporate the limitations of achieving the absence of effective CD4 T cell help by including prior to or in step c, at least one agent that inhibits or eliminates effective CD4 T cell help. The Examiner alleges that while we had pointed out text by page and line number to support this amendment, the Examiner alleges that said text describes exposure of a co-culture of dendritic cells and apoptotic cells, and therefore does not provide support for the limitation of "prior to" step c.

Applicants respectfully traverse the Examiner's rejection and have further amended the claim to better describe the invention. More particularly, claim 4 has been amended to read:

"The method of claim 1 wherein said agent that inhibits or eliminates effective CD4+ T cell help is removed from the dendritic cells prior to exposure of the dendritic cells to the T cells."

Applicants assert that the support for this amendment to claim 4 can be found on page 45, lines 21-23, continuing onto page 46, line 1. In particular, the Examiner's attention is drawn to this page, whereby Applicants state:

"This however, is not the mechanism by which the FK506 is blocking the activation of T cells via the cross-presentation pathway, as residual drug is removed prior to the DCs being added to the T cells (Figure 9C)."

Further in that same paragraph, Applicants state:

"No residual FK506 remained in the co-culture to inhibit T cell activation (Figure 9C)...)

Applicants assert that the amendment to claim 4 obviates the Examiner's rejection under 35 U.S.C. §112, first paragraph.

Rejection under 35 U.S.C. §103(a)

In addition, the Examiner has rejected claims 1, 2, 19, 21, 22 under 35 U.S.C. §103(a) as being unpatentable over Albert, *et al.*, (Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368, cited in a previous Office Action) in view of Albert, *et al.*, (Nature, 1998, Vol. 392, pp. 86-89) and Heath, *et al.*, (Journal of Experimental Medicine, 1998, vol. 1897, pp. 1549-1553.

The Examiner notes that the specific embodiments of the present claims are recited in the previous Office Action.

Albert, *et al.*, Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368

The Examiner alleges that Albert, *et al.*, (JEM) teach that dendritic cells phagocytose apoptotic cells and cross present antigens from the apoptotic cells to cytotoxic T-lymphocytes (abstract). The Examiner further alleges that Albert, *et al.*, teach that dendritic cells can acquire antigens from tumors, transplants, infected cells and self tissues for stimulation or tolerization of CTLs (abstract), thus fulfilling the specific limitation of claim 19 specifying the types of antigen for which tolerance might be evoked. Furthermore, the Examiner alleges that Albert, *et al.*, teach

the isolation of dendritic cells from peripheral blood and the use of monocyte conditioned medium (MCM) as a maturation factor for the dendritic cells, thus fulfilling the specific embodiments of claim 2 (page 1360, first column, lines 12-14 under the heading of "Preparation of Cells"). In addition, the Examiner alleges that Albert, *et al.*, also teach that on days 10 and 11, the cells were of the mature phenotype CD14-, CD83+ and HLA-DRhi (Page 1360, first column, lines 15-17 under the heading "Preparation of Cells"). In addition, the Examiner alleges that Albert, *et al.*, also teach the maturation factors of LPS, ceramide, CD40L, TNF-alpha and PGE2 in addition to macrophage conditioned medium (page 1359, second column, lines 13-17). In addition, the Examiner alleges that Albert, *et al.*, teach that the co-culture of immature dendritic cells with apoptotic cells in the presence of macrophage conditioned medium, a maturation stimulus for dendritic cells, made apoptotic cells an even better target for cross-presentation of antigen (page 1362, second column, lines 4-8).

The Examiner notes that Albert, *et al.*, do not teach the induction of tolerance by the exposure of dendritic cells to apoptotic cells in the absence of effective CD4 T cell help.

Albert, *et al.*, Nature, 1998, Vol. 392, pp. 86-89

The Examiner alleges that Albert, *et al.*, (Nature) teach the cross-priming of T-cells via apoptotic cells which are phagocytosed by dendritic cells (page 88, second column, lines 27-35). The Examiner further alleges that Albert, *et al.*, teach that tolerance to an antigen may be dependent upon apoptotic cell death followed by antigen presentation by dendritic cells (page 88, second column, lines 35-38, 40-42). The Examiner also alleges that Albert, *et al.*, conclude that the apoptosis dependent pathway has the potential to be manipulated to modulate the immune response (page 88, second column, lines 43-46).

Heath, *et al.*, Journal of Experimental Medicine, 1998, vol. 187, pp. 1549-1553

The Examiner alleges that Heath, *et al.*, teach that a number of experimental models have revealed that CD4 T cell help is important for the induction of CTL (page 1551, second column, lines 29-33). In addition, the Examiner alleges that Heath, *et al.*, propose that CD4 T cell help determines if a T cell which has been exposed to a dendritic cell presenting antigen will be "cross tolerized" versus "cross-primed" (page 1551, second column, line 12 and lines 33-35).

The Examiner alleges that it would have been *prima facie* obvious at the time the invention was made to expose the dendritic cells to apoptotic cells in the absence of effective T cell help in order to induce tolerance to the antigens made available by the apoptotic cell. The Examiner further alleges that one of skill in the art would have been motivated to do so by the teachings of Albert, *et al.*, (Nature) on the induction of a tolerogenic response by presentation of apoptotic cells by dendritic cells, and the teachings of Heath, *et al.*, who suggest that lack of CD4 T cell help can result in cross-tolerization to antigens from apoptotic cells taken up by dendritic cells.

Moreover, the Examiner has noted that Applicants have argued that the above combination is not the claimed invention, because the above combination relies on the apoptosis of T cells after activation by the dendritic cells, whereas the instant invention relies on the direct interaction between the dendritic cell and the T cell. The Examiner has noted that only previously presented claim 4 requires that the agent for elimination of effective CD4 T cell help be eliminated from the dendritic cell before exposure to the T cells.

Applicants' position

The Examiner fails to set forth a proper *prima facie* case of obviousness

Applicants remind the Examiner that a rejection under 35 U.S.C. §103 is proper only when a prior art reference alone or in combination with a second prior art reference renders the invention obvious. Applicants further remind the Examiner that a rejection based upon a combination of references is not proper unless the following three criteria are met: 1) the references in combination teach every single element of the invention as claimed; 2) there must be some suggestion or motivation in the prior art to combine the references to reach the invention as claimed; and 3) there must be a reasonable expectation of success in making the combination to reach the invention as claimed.

Any alleged combination of references in the present situation fails the most basic test for the appropriateness of a rejection. The Examiner admits that Albert *et al.* do not teach the induction of tolerance by the exposure of dendritic cells to apoptotic cells in the absence of effective CD4 T cell help. Rather, the present invention teaches a previously undiscovered

means of inducing tolerance in the presence of CD4 T cells, by treatment with a drug such as FK506 or rapamycin, that blocks CD4 T cell help.

In addition, as shown below by Applicants' representatives, the remaining references simply cannot be combined in any way to result in the subject matter as currently claimed in the present application.

The present invention as claimed. The claims of the present application are drawn to methods for inducing tolerance in a mammal to an antigen comprising the steps of: isolating peripheral blood mononuclear cells (PBMC) from a whole blood sample from said mammal; isolating dendritic cells from said PBMC; **exposing said dendritic cells *ex vivo* to apoptotic cells expressing said antigen** in the presence of at least one dendritic cell maturation stimulatory molecule and in the absence of effective CD4+ T cell help, **wherein the absence of effective CD4+ T cell help is achieved by treating the dendritic cells with an agent that inhibits or eliminates effective CD4+ T cell help**; and **introducing the dendritic cells into said mammal**; wherein said dendritic cells induce apoptosis of antigen-specific CD8+ T cells in said mammal resulting in tolerance to said antigen. To clarify, by *the absence of effective CD4+ T cell help* the Applicants do not mean the absence of CD4+ T cells; rather the present invention teaches a means of inducing tolerance in the presence of CD4 T cells (as might be expected in a living mammal), by treatment with a drug such as FK506 or rapamycin, that blocks CD4 T cell help from being *effective*. The dependent claims are drawn to particular dendritic cell maturation factors including PGE2, TNF-alpha, lipopolysaccharide, monocyte conditioned medium, CpG-DNA, or any combination thereof. Additional dependent claims are drawn to methods for exclusion of effective CD4+ T cell help by including at least one agent that inhibits or eliminates effective CD4+ T cell help. Furthermore, **the agent that inhibits or eliminates effective CD4+ T cell help is used to treat the dendritic cell and is washed out prior to exposure of the dendritic cell to the T cell.** In addition, the agent which inhibits or eliminates effective CD4 T cell help inhibits signaling consequent to dendritic cell-CD4 T cell engagement. Such an agent is selected from a FKBP antagonist and a TOR antagonist. The FKBP antagonist is tacrolimus. The TOR antagonist is rapamycin. The antigen is a tumor antigen, a viral antigen, a self-antigen

or a transplant antigen. The dendritic cells are infused into the mammal after the dendritic cells mature and the mammal is preferably a human.

Applicants' Position Regarding Albert et al. (Journal of Experimental Medicine, 1998, Vol. 188, pp. 1359-1368)

Albert, *et al.*, (JEM) teach that dendritic cells phagocytose apoptotic cells and cross present antigens from the apoptotic cells to cytotoxic T-lymphocytes (abstract). Albert, *et al.*, also teach that dendritic cells can acquire antigens from tumors, transplants, infected cells and self tissues for stimulation or tolerization of CTLs (abstract). Furthermore, Albert, *et al.*, teach the isolation of dendritic cells from peripheral blood and the use of monocyte conditioned medium (MCM) as a maturation factor for the dendritic. In addition, Albert, *et al.*, also teach that on days 10 and 11, the cells were of the mature phenotype CD14-, CD83+ and HLA-DRhi. In addition, Albert, *et al.*, teach the maturation factors of LPS, ceramide, CD40L, TNF-alpha and PGE2 in addition to macrophage conditioned medium and also teach that the co-culture of immature dendritic cells with apoptotic cells in the presence of macrophage conditioned medium, a maturation stimulus for dendritic cells, made apoptotic cells and even better target for cross-presentation of antigen.

Applicants assert that Albert *et al.* **do not teach or suggest** the methods of the present invention for inducing tolerance as currently claimed. More particularly, Applicants assert that Albert, *et al.*, **do not teach the induction of tolerance by the exposure of dendritic cells to apoptotic cells in the absence of effective CD4 T cell help, wherein the absence of effective CD4+ T cell help is achieved by treating the dendritic cells with an agent that inhibits or eliminates effective CD4+ T cell help.**

Moreover, Applicants assert that Albert *et al.* **do not teach or suggest that the absence of CD4+ T cell help, or the inhibition of CD4+ T cell help through the use of inhibitors of signaling consequent to dendritic cell-CD4+ T cell engagement** using the methods described in the present application for tolerance induction. In addition, Albert *et al.* **do not teach or suggest the inhibition of signaling using agents such as those described herein, such as FK506 or rapamycin**. More particularly, Albert *et al.* **do not teach or suggest that the agents which inhibit signaling, such as those described in the present application, including FK506**

or rapamycin, are used to treat the dendritic cell, and are washed out prior to exposure of the dendritic cell to the T cell. Applicants have shown in the present application that pre-treatment of the dendritic cell with such an agent, followed by a wash-out of the agent prior to addition or exposure of the dendritic cells to the T cells, results in tolerization.

Applicants' Position Regarding Albert, *et al.*, Nature, 1998, Vol. 392, pp. 86-89

Albert, *et al.*, (Nature) teach the cross-priming of T-cells via apoptotic cells which are phagocytosed by dendritic cells. Albert, *et al.*, teach that tolerance to an antigen may be dependent upon apoptotic cell death followed by antigen presentation by dendritic cells.

Applicants assert that Albert *et al.* **do not teach or suggest** the methods of the present invention for inducing tolerance as currently claimed. More particularly, Applicants assert that Albert, *et al.*, **do not teach or suggest the induction of tolerance by the exposure of dendritic cells to apoptotic cells in the absence of effective CD4 T cell help, wherein the absence of effective CD4+ T cell help is achieved by treating the dendritic cells with an agent that inhibits or eliminates effective CD4+ T cell help.**

Moreover, Applicants assert that Albert *et al.* **do not teach or suggest the absence of CD4+ T cell help, or the inhibition of CD4+ T cell help through the use of inhibitors of signaling consequent to dendritic cell-CD4+ T cell engagement** using the methods described in the present application for tolerance induction. In addition, Albert *et al.* **do not teach or suggest the inhibition of signaling using agents such as those described herein, such as FK506 or rapamycin**. More particularly, Albert *et al.* **do not teach or suggest that the agents which inhibit signaling, such as those described in the present application, including FK506 or rapamycin, are used to treat the dendritic cell, and are washed out prior to exposure of the dendritic cell to the T cell.** Applicants have shown in the present application that pre-treatment of the dendritic cell with such an agent, followed by a wash-out of the agent prior to addition or exposure of the dendritic cells to the T cells, results in tolerization.

Applicants' Position regarding Heath, *et al.*, Journal of Experimental Medicine, 1998, vol. 187, pp. 1549-1553

Heath, *et al.*, teach that a number of experimental models have revealed that CD4 T cell help is important for the induction of CTL (page 1551, second column, lines 29-33). In addition, Heath, *et al.*, propose that CD4 T cell help determines if a T cell which has been exposed to a dendritic cell presenting antigen will be "cross tolerized" versus "cross-primed".

Applicants assert that Heath *et al.* **do not teach or suggest** the methods of the present invention for inducing tolerance as currently claimed. More particularly, Applicants assert that Heath, *et al.*, **do not teach or suggest the induction of tolerance by the exposure of dendritic cells to apoptotic cells in the absence of effective CD4 T cell help, wherein the absence of effective CD4+ T cell help is achieved by treating the dendritic cells with an agent that inhibits or eliminates effective CD4+ T cell help.**

Moreover, Applicants assert that Heath *et al.* **do not teach or suggest the absence of CD4+ T cell help, or the inhibition of CD4+ T cell help through the use of inhibitors of signaling consequent to dendritic cell-CD4+ T cell engagement** using the methods described in the present application for tolerance induction. In addition, Heath *et al.* **do not teach or suggest the inhibition of signaling using agents such as those described herein, such as FK506 or rapamycin**. More particularly, Heath *et al.* **do not teach or suggest that the agents which inhibit signaling, such as those described in the present application, including FK506 or rapamycin, are used to treat the dendritic cell, and are washed out prior to exposure of the dendritic cell to the T cell.** Applicants have shown in the present application that pre-treatment of the dendritic cell with such an agent, followed by a wash-out of the agent prior to addition or exposure of the dendritic cells to the T cells, results in tolerization.

The Argument for Non-obviousness based on the combined teachings of Albert *et al.* (JEM) in view of Albert *et al.* (Nature) and Heath *et al.*

Applicants assert that the reference of Albert *et al.* (JEM) in view of Albert *et al.* (Nature) and Heath *et al.*, when considered alone or in combination, do not teach or suggest the subject matter as presently claimed in the present application for inducing tolerance by exposing dendritic cells to antigen in the context of an apoptotic cell ***ex vivo***, in the presence of at least one

dendritic cell maturation stimulatory molecule and in the absence of effective CD4+ T cell help, wherein the absence of effective T cell help is achieved by pre-treating the dendritic cell with an agent that prevents subsequent signaling when the dendritic cell is exposed to or comes into contact with a T cell. More importantly, **none of the references cited** takes into account the unexpected findings of Applicants, **whereby tolerization can be achieved by treating the dendritic cell with such an agent as those described in the present application, followed by washing out the agent prior to exposure of the dendritic cell to the T cell**. While Applicants believe that the teachings of Albert *et al.* (JEM) do not extend to the methods of inducing tolerance as described in the instant application and in the currently amended claims, Applicants further assert that even if Albert *et al.* were a proper reference under 35 U.S.C. §103(a), there would still be no reasonable expectation of success, when combined with Albert *et al.* (Nature) and Heath *et al.* More particularly, Applicants assert that neither of the Albert *et al.* references nor the Heath *et al.* reference teach or suggest that dendritic cells, when exposed ***ex vivo*** to antigen presented in the context of an apoptotic cell, and in the presence of agents that induce maturation of the dendritic cell but in the absence of T cell help, wherein the absence of T cell help is achieved by **treating the dendritic cells with an agent such as those described herein and washing out the agent prior to exposure of the dendritic cell to the T cell,** followed by reintroduction of these dendritic cells to a mammal, results in apoptosis of antigen specific T cells.

Moreover, it is Applicants' belief that the Examiner is misunderstanding the basic concept of the invention in that one of the mechanisms used to block T cell help is through **use of an agent such as FK506 or rapamycin for treating the dendritic cell with such agent, not the T cell. Applicants are not inducing apoptosis of the antigen specific T cell by treating the T cell with FK506 or rapamycin. Applicants are treating the dendritic cell with FK506 or rapamycin, followed by a wash-out of the drug prior to reintroducing the dendritic cells to the mammal.** Support for this can be found in the instant application on page 45, lines 21-23, continuing on to page 46, lines 1-4.

In summary, it is Applicants' assertion that any rejection based on Albert *et al* (JEM) alone or in combination with Albert *et al.* (Nature) and Heath *et al.* fails for the following reasons:

1. There simply is no teaching or suggestion of tolerance induction by presenting antigen to dendritic cells *ex vivo* via apoptotic cells in the presence of dendritic cell maturation factors, but in the absence of CD4+ T cell help, **wherein the absence of T cell help is achieved using an agent to treat dendritic cells**, and wherein said agent inhibits or eliminates effective CD4+ T cell help such that transfer of the dendritic cells to a subject results in apoptosis of antigen specific CD8+ T cells.
2. Furthermore, there is no teaching or suggestion that the absence of CD4+ T cell help could be substituted **by treating the dendritic cells with an agent such as FK506 or rapamycin, and washing out the drug prior to exposure of the dendritic cells to T cells.**
3. Furthermore, there is no teaching or suggestion that **the dendritic cells** could be treated with an agent such as FK506 or rapamycin ***ex vivo*** followed by washing out the drug prior to readministration of the dendritic cells to a mammal, wherein said dendritic cells induce apoptosis of antigen-specific CD8+ T cells in said mammal, resulting in tolerance to the antigen.

The analysis under § 103(a). Thus, Applicants assert that the references cited above, Albert *et al* (JEM) in view of Albert *et al* (Nature) and Heath *et al*, when used alone or in combination with each other **do not teach or suggest** the methods disclosed in the present application for tolerance induction. Moreover, since the methods described in the present application for tolerance induction were unknown at the time of the references cited, most particularly, **the use of an agent to treat dendritic cells prior to addition to T cells, or prior to readministration to a mammal**, it was not possible to predict the steps and conditions necessary to optimize induction of antigen specific tolerance. Moreover, it was not until Applicants' present invention that the precise steps involved in tolerance induction by presenting antigen to dendritic cells via apoptotic cells ***ex vivo*** in the presence of dendritic cell maturation factors, but in the absence of CD4+ T cell help, wherein the absence of T cell help was achieved by treating the dendritic cells with an agent such as those described in the present application, such as FK506, washing out the agent, and readministering the dendritic cells to a mammal, wherein such administering resulted in apoptosis of antigen specific CD8+ T cells, were identified. Furthermore, it was not until the

time of Applicants' own research that it was realized that the absence of CD4+ T cell help could be substituted by first **treating the dendritic cell with an inhibitor of signaling, such as with FK506 or rapamycin, then washing out the inhibitor prior to exposure to the T cell**. In addition, the present invention teaches, and no prior art suggests, that a specific action of such inhibitors of signaling on a reconstituted system consisting of dendritic cells and apoptotic cells, **provides a long-lasting effect on those dendritic cells such that they are now "inhibitory/tolerizing" even with subsequent encounter to CD4.**

In light of the foregoing claim amendments and arguments, Applicants respectfully request withdrawal of the rejection.

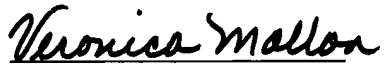
Fees

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

Conclusion

Applicants believe that in view of the foregoing, the claims are in condition for allowance. Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,



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